Method for Full-Length cDNA Cloning Using Degenerate Stem-Loop Annealing Primers

Abstract of the Disclosure

The present invention provides a method for isolating a double-stranded cDNA having a nucleotide sequence of a complete open reading frame which comprises: (A) admixing (i) an isolated single-stranded cDNA, (ii) a first primer capable of forming a stem-loop structure, comprising (a)at the 3' end of the primer, a first random sequence, linked to (b) a second sequence, linked to (c) a third sequence which forms a loop structure, linked to (d) a fourth sequence, at the 5' end of the first primer, which is complementary to the second sequence, under hybridization conditions sufficient for annealing the first sequence of the first primer to the sequence at the 3' end of the single-stranded cDNA, and (iii) a polymerase; (B) incubating the mixture from step (A) under suitable conditions for DNA synthesis; and (C) performing a polymerase chain reaction by admixing (i) an aliquot of the mixture from (B), (ii) a second primer which specifically binds to the single-stranded cDNA, (iii)a third primer which comprises (a) a fifth sequence identical to the third sequence of the first primer, linked to (b) a sixth sequence identical to a portion of the second sequence of the first primer, and (iv) a polymerase, under conditions suitable for a polymerase chain reaction so as to produce a double-stranded cDNA reaction product, thereby isolating the cDNA having the sequence of the complete open reading frame.